the basis of significant epidermal hyperplasia, dyskeratosis, dermal fibrosis with hemorrhage, loss of hair follicles and sebaceious glands, inflammation of subcutaneous fat tissue and hemorrhagic cysts. These findings confirmed that GvHD could be induced using the macaque model. The third animal was transplanted with haploidentical donor bone marrow (1.1×10^9 total nucleated cells/kg and 6.9×10^7 CD3+ T cells/kg), but was given T cell costimulation blockade with abatacept (targeting the CD28/B7 pathway) and an anti-CD40 antibody (targeting the CD40/CD154 pathway) in addition to rapamycin. Abatacept is currently clinically approved for treatment of rheumatoid arthritis, but has not been added to clinical GvHD regimens, largely due to the paucity of translational efficacy data. This animal maintained full donor chimerism (including 100% T and B cell chimerism), until day 63 when he was sacrificed, without signs of GvHD. Additional animals are now being added to the study, to confirm these initial results.

Conclusions: 1. We have established a robust non-human primate model of GvHD using pedigreed and MHC-typed rhesus macaques. 2. We have used this model to begin to test the efficacy of a novel agent combination, capable of preventing the onset and complications of acute GvHD. 3. Preliminary results suggest that CD28- and CD40-directed costimulation blockade may be active agents for the prevention of GvHD. A large scale analysis of their efficacy and immune consequences is currently underway.

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EVALUATION OF OPTIMAL BLOOD CONCENTRATION OF TACROLIMUS FOR THE PROPHYLAXIS OF ACUTE GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM UNRELATED DONOR

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Background: Optimal blood concentration of tacrolimus for the prophylaxis of GVHD after allogeneic hematopoietic stem cell transplantation (HSCT) has not been established. Our retrospective analysis has shown that incidence of Grades II-IV acute GVHD in patients with mean tacrolimus concentration less than 15 ng/ml was significantly higher than that in patients with mean tacrolimus concentration 15 ng/ml or higher. The present study has set the target tacrolimus concentration between 15 and 20 ng/ml, and evaluated the efficacy in preventing the development of acute GVHD after allogeneic HSCT from unrelated donor.

Patients and Methods: Patients undergoing allogeneic HSCT from an HLA-serologically matched unrelated donor for hematological diseases were evaluated. Stem cell source was bone marrow in all patients. For the prophylaxis of GVHD, tacrolimus and methotrexate were administered. Tacrolimus was given at an initial dose of 0.03 mg/kg by continuous intravenous infusion from day -1, and its dose was arranged to maintain its blood level at 15–20ng/ml during the first 4 weeks after HSCT. MTX was given at a dose of 15 mg/m2 on day 1, and 10 mg/m2 on days 3, 6, and 11.

Results: Of the 55 evaluated patients, 20 (36.4%) patients developed grade II, 2 patients (3.6%) developed grade III, and no patient developed grade IV acute GVHD. In multivariate analysis, HLA allele mismatch was an only identified risk factor for developing grades II-IV acute GVHD, while blood concentration of tacrolimus was not. No serious complications resulting in discontinuation of tacrolimus were observed.

Conclusions: Tacrolimus combined with methotrexate could effectively prevent the development of severe acute GVHD after allogeneic HSCT from unrelated donor by maintaining its blood concentration between 15 and 20 ng/ml in the early post-transplant period.

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VISUALIZING THE FATE OF FOXP3 PROTEIN IN HUMAN T-CELLS

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Foxp3 plays a pivotal role in the development and function of regulatory T cells (Treg) which represent a promising tool for transplantation tolerance induction. In murine but not human T cells, forced expression of Foxp3 converts naive T cells into functional Tregs. This might in part be due to the fact that Foxp3 protein expression is difficult to track because endogenous Foxp3 is also expressed in activated human T-cells. We therefore intended to track the fate of ectopically expressed Foxp3 protein in human CD4+ cells with the aim of defining why forced Foxp3 overexpression was not sufficient for gaining regulatory function and to learn about the physiological regulation of Foxp3 protein in human T cells. We used a non-viral nucleofection protocol for transfection of human CD4+ cells with a dual-expression plasmid of a GFP-Foxp3 fusion protein and a truncated low affinity nerve growth factor receptor (LNGFR) to allow for positive selection of transfected cells. As expected we could demonstrate that purified, transiently Foxp3 transfected cells only weakly suppressed proliferation of T cells. We found that the ectopically expressed Foxp3 protein disappeared more quickly than Foxp3 mRNA levels which remained at high levels for up to 4 days. Also in stably transfected T cell lines GFP-Foxp3 mRNA but not the fusion protein was detectable. We hypothesized that this might be due to posttranslational protein degradation or posttranscriptionally decreased protein synthesis. We observed appearance of Foxp3 protein in stably transfected Jurkat cells treated with the proteasome inhibitor bortezomib implying ubiquitinylation as one mechanism of Foxp3 protein degradation. By fluorescent microscopy we could demonstrate that Foxp3 was relocated from the nucleus to the cytoplasm within 24 hours after transfection. Using a plasmid encoding for a Foxp3 protein double-tagged with N-terminal GFP and C-terminal YFP we found that the GFP tagged fragment was relocated to the cytoplasm and disappeared after 4 days, while the YFP tagged fragment remained in the nucleus. Both a nuclear import and a nuclear export motif in the coding sequence of Foxp3 could be identified. Our findings demonstrate that human Foxp3 protein expression is subject to a regulatory pathway controlling subcellular distribution as well as protein stability. This experimental setup should provide a basis for further conclusions about the natural biology of this important determinator of Treg function.

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HIGH BAFF:B CELL RATIOS AND CIRCULATING ACTIVATED B CELLS IN CHRONIC GVHD

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Although rituximab is an effective treatment for steroid refractory chronic GVHD (cGVHD), the mechanisms underlying B cell involvement in cGVHD have not been elucidated. Previous studies have demonstrated that patients with active cGVHD have high levels of BAFF, a pivotal B cell survival cytokine. We hypothesized that B cell reconstitution in the context of high BAFF after HSCT could support activated, potentially pathologic B cell populations that are most dependent on BAFF for survival. We first performed detailed phenotypic and functional analyses of peripheral B cells in 57 patients >12 month post-HSCT and 33 healthy controls (see table).

Patient Group (median month post-HSCT)	BAFF: B Cell Ratio (ng BAFF/ 1000 B cells)	p-value vs. no cGVHD	Median Naive B Cell Number (×1000/L)	p-value vs. no cGVHD	Median Transitional B Number (×1000/L)	p-value vs. no cGVHD
Healthy (no-HSCT) n=33	0.008	0.48	89.5	0.0004	14.4	0.02
No cGVHD (27 mo.) n=12	0.008		260.5		28.7	
Inactive cGVHD (31 mo.) n=23	0.026	0.01	99.1	0.0005	8.5	0.04
Active cGVHD (21 mo.) n=22	0.046	<0.0001	79.8	0.0009	8.1	0.04